Agriculture, Ecosystems and Environment 268 (2018) 15–23

Contents lists available at ScienceDirect

Agriculture, Ecosystems and Environment

journal homepage: www.elsevier.com/locate/agee

High carbon storage in a previously degraded subtropical soil under no-tillage with legume cover crops

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ARTICLE INFO

Keywords:
Carbon sequestration
Soil depth
Conservation agriculture
Cropping system
Climate change mitigation

ABSTRACT

The effect of no-tillage (NT) on soil organic carbon (SOC) storage may help Brazil meet its 37% greenhouse gas emissions reduction target by 2025. When combined with legume cover crops, NT could result in even greater SOC storage than NT alone. The objective of this study was to evaluate the SOC storage potential of NT and the contribution of legume cover crops and nitrogen (N) fertilization to this potential in both the surface and subsurface soil layers of a previously degraded subtropical Acrisol of Southern Brazil. Using a split-plot design, the long-term field experiment compared the effect of NT and conventional tillage (CT), with or without legume cover crops, and with or without mineral N fertilization. Thirty years of contrasting management systems resulted in large differences (up to 35 Mg ha^{-1}) in SOC stocks in the whole soil profile (0–100 cm). The combination that provided the greatest increase in SOC was NT combined with two legume cover crops and N fertilization (1.15 Mg ha^{-1} year^{-1} compared to CT, with no N fertilization or legume cover crop). Legume cover crops were twice as efficient in storing SOC as N fertilization, with 1 kg of residue input being converted to 0.15 kg of SOC. Overall, the variation in SOC stocks was explained largely by plant carbon input (R^2 = 80%) which varied with N fertilization and cropping system. About half of the SOC storage that occurred in this 30-year-old NT system was attributable to the increase in SOC stocks in the subsurface layer (30–100 cm), which was confirmed by the contribution of C_3 cover crop residues using carbon isotope signature (from 14.8 to ~17.5‰ in the 75–100 cm layer). Thus, the legume cover crop made a strong contribution to the potential of SOC storage in NT, and high rates of C storage occurred over a longer period in subsurface soil layers than previously believed.

1. Introduction

The Paris climate agreement is aimed at holding global warming to below 2 °C by 2050 and to pursue efforts to limit it to 1.5 °C (Rogelj et al., 2016). Brazil made a voluntary commitment to reduce greenhouse gas (GHG) emissions by 37% by 2025 and established a climate plan, which encompasses a low carbon agriculture plan which includes NT farming as one of five thrusts for mitigation of GHG emissions (Brazil Ministry of Environment, 2015).

In the last century, Brazilian agriculture has revolved around conventional tillage practices that have led to severe soil degradation problems including water erosion and the loss of soil quality (Mielniczuk et al., 2003). In this context, the no-tillage (NT) system emerged as a basis for conservation and sustainable agriculture (Paustian et al., 1997; Bayer et al., 2000; Lal et al., 2007), with strong impact on chemical, physical and biological soil quality (Mielniczuk et al., 2003). Studies conducted in tropical and subtropical environments (Bayer et al., 2006) have highlighted mean annual SOC storage rates ranging from 0.35 to 0.48 Mg ha^{-1}yr^{-1} when conventional tillage systems are converted into NT systems.

The effect of NT on SOC storage is also dependent on the amount and diversity of the crops grown (Diekow et al., 2005; Martins et al., 2012; Raphael et al., 2016). Legume cover crops play an essential role in SOC storage under NT, either through the biomass inputs (shoot and root) associated with these plant species or through the symbiotically fixed N which becomes available and increases the grain and biomass

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https://doi.org/10.1016/j.agee.2018.08.024
Received 25 October 2017; Received in revised form 22 August 2018; Accepted 28 August 2018
Available online 08 September 2018
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production of cash crops grown in succession (Amado et al., 2006). The higher quality (N content and soluble fractions) of the biomass of legume cover crops, whether cultivated alone or intercropped with other species, may improve the efficiency of the microorganisms to accumulate C in the soil (Cotrufo et al., 2013). However, there is no consensus on the effect of nitrogen fertilization on soil SOC stocks because, in spite of the positive effect it has on plant biomass addition and consequent C input (Mack et al., 2004, Kirchmann et al., 2013), inorganic N can also cause accelerated mineralization (Khan et al., 2007) although this conclusion is not universally accepted (e.g., Powlson et al., 2010).

Soil depth should be considered carefully in evaluating SOC storage under NT. In temperate soils of North America and Europe, the gain of SOC in surface layer under NT may be compensated by gains in subsurface layers under CT (Angers et al., 1997; Baker et al., 2007; Blanco-Canqui et al., 2011; Dimassi et al., 2014). By contrast, some studies carried out in tropical and subtropical soils in Brazil have shown that sampling of the surface soil can lead to underestimation of the potential of SOC storage under NT (Boddey et al., 2010; Alburquerque et al., 2015). In those studies, sampling of the 0–100 cm layer resulted in SOC storage rates that were 59% (Boddey et al., 2010) and 100% (Alburquerque et al., 2015) higher than for soil sampling done at a depth of 0–30 cm. The storage of C in subsurface layers under NT may be significant in Brazilian tropical and subtropical soils, especially in the case of cropping and rotation systems that incorporate legume cover crops (Boddey et al., 2010). The high volume of rainfall may favor the percolation of organic compounds and contribute to the potential for SOC storage in subsurface layers of tropical soils under conservation management systems (Hobley and Wilson, 2016). In addition, free-draining soils (Miranda et al., 2016) with Bt horizons (Torres-Sallan et al., 2018) and functional groups on the surface of iron and aluminum oxides that interact strongly and stabilize organic matter (Lawrence et al., 2015) point to considerable potential for SOC storage in subsurface layers.

We hypothesized that over the long term, legume cover crops would make a strong contribution to the potential of SOC storage under NT, and that C storage could occur at high rates in subsurface layers of subtropical soils. Our objective was thus to evaluate the potential that NT offers for SOC storage as well as the contribution of legume cover crops and N fertilization to this potential in both surface and subsurface layers of a previously degraded subtropical Acrisol.

2. Materials and methods

2.1. Description of the experiment

The study was conducted in a long-term experiment (30 years) at the Agronomic Experimental Station of the Federal University of Rio Grande do Sul in the municipality of Eldorado do Sul–RS (30º06 ′ S, 51º40 ′ W, elevation 96 m). The climate is subtropical (Cfa according to the Köppen classification), with a mean temperature of 19.4 °C and annual rainfall of 1440 mm. The soil was classified as a sandy clay loam granite-derived Acrisol (FAO, 2002), with a loamy clay texture in the surface layer. The clay content in the soil profile increases from 217 g kg^{-1} in the 0–5 cm layer to 394 g kg^{-1} in the 20–30 cm layer, reaching 511 g kg^{-1} in the 75–100 cm layer. The main minerals in the clay fraction are kaolinite (720 g kg^{-1}) and iron oxides (109 g kg^{-1}) (Bayer et al., 2001).

Prior to this experiment, the field was a natural grassland (mainly *Paspalum* spp. and *Andropogon* spp.), which was converted to cropland in 1969 and cultivated for 16 years by using conventional tillage practices based on plowing and disking twice a year for winter and summer annual crops with straw removal. When the experiment was started in 1985, the soil showed serious physical degradation and water erosion (Bayer et al., 2000).

The experiment included two soil tillage systems (CT and NT) arranged in main plots of 15 × 20 m. Each tillage system was composed of three cropping systems in subplots of 5 × 20 m: black oats (*Avena strigosa* Schreb)/maize (*Zea mays* L.) (O/M), vetch (*Vicia sativa* L.)/maize (V/M) and oats + vetch/maize + cowpea (*Vigna unguiculata* (L.) Wald) (OV/MC). These combined tillages and cropping systems were managed with two levels of fertilization, 0 and 180 kg ha^{-1} of N-urea (0 N and 180 N), applied in strips in the maize crop only, consisting the sub-subplots (5 × 10 m). The experimental design consisted of randomized blocks with split-split plots and three replicates.

Winter crops, managed as cover crops, were established in April–May of each year using direct drilling in both CT and NT treatments. Oats, when grown alone, were seeded at a rate of 80 kg ha^{-1}. When oats were grown with vetch, oats were seeded at 30 kg ha^{-1}, and vetch at 50 kg ha^{-1}. For vetch cultivated alone, 80 kg ha^{-1} was used. In the OV/MC system, cowpea was sown 15–20 days after the maize, between the lines of this crop which were 40 cm apart.

The CT plots were ploughed to a furrow-depth of 17 cm once a year in spring before maize sowing by using a three-disk plough and harrowed twice to a depth of 10 cm by using a disk harrow resulting in the incorporation of the crop residues in this layer. At the same time, glyphosate-based herbicide (Roundup, Monsanto) was applied in the NT plots at a 1.4 kg ha^{-1} rate relative to the final glyphosate concentration, and 2–3 days later the winter cover crops were managed with a knife-roller and aboveground residues left on the soil surface. In NT, soil disturbance occurred only in the sowing line and the residues of the cover crop were left on the soil surface.

Maize was planted in September–October, with between-row spacing of 90 cm and a sowing rate designed to obtain 50–70 thousand plants per hectare. The fertilizer rate applied in maize was 21.5 and 41.5 kg ha^{-1} of P and K (50 and 50 kg ha^{-1} of P_2O_5 and K_2O), respectively.

The mean annual C input (aboveground and root, with roots being assumed to account for 30% of the aboveground portion) was calculated from cover crop data and the dry-matter maize yields compiled by Zanatta et al. (2007) for the period 1985–2006, which were subsequently updated to 2014 (Fig. 1). The annual aboveground maize dry matter yield was estimated from grain yield, and aboveground C input calculated by assuming dry matter in maize and the cover crops to contain 40% C. The values of CT and NT were averaged due to similar C input in both tillage systems.

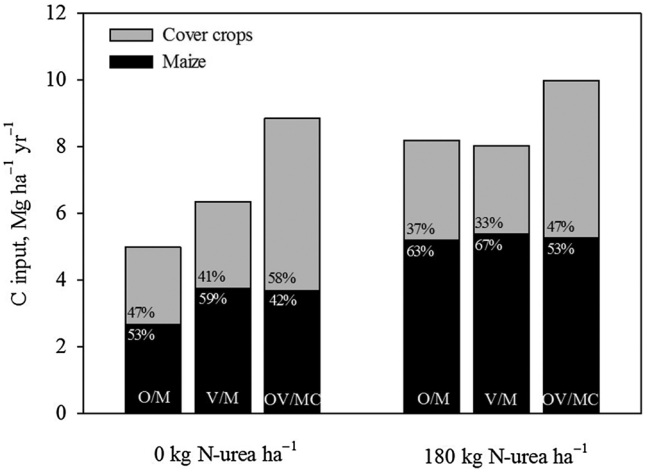

Fig. 1. Mean annual C inputs for oat/maize (O/M), vetch/maize (V/M) and oat + vetch/maize + cowpea (OV/MC) cropping systems subjected to two N-urea rates (0 N = 0 kg ha^{-1} and 180 N = 180 kg ha^{-1}). Values are average of two tillage systems (no-tillage and conventional tillage).

2.2. Soil bulk density and SOC concentrations and stocks

Soil samples from eight layers (0–5, 5–10, 10–15, 15–20, 20–30, 30–50, 50–75 and 75–100 cm) were collected in September 2014 prior to soil tillage for maize crop establishment. Trenches were excavated with a backhoe to allow measurement of soil bulk density in duplicate by the volumetric ring method (Blake and Hartge, 1986) only in sub-subplots with no nitrogen fertilization, assuming that nitrogen fertilization did not modify soil density.

Soil samples for determining SOC concentration were obtained from the 0 and 180 N sub-subplots, using the same layers as for bulk density samples. Two sub-samples per plot were collected with a spiral auger (Φ = 25 cm) that were air dried and ground to ≤2 mm in a Marconi 330 grinder, a subsample of ~2 g being further ground to ≤250 µm in an agate mortar to determine SOC by dry combustion in a FlashEA 1112 instrument from Thermo Electron Corp. (Milan, Italy).

SOC stocks down to depths of 30 and 100 cm were calculated using the equivalent soil mass approach (Ellert and Bettany, 1995), which considers equal masses of soil between treatments. The soil under CT O/M 0N was used as the reference for the equivalent mass. The annual SOC storage rate was calculated as the difference between the SOC stocks of the treatments and the reference system, divided by the time elapsed since the implementation of the experiment, i.e. 30 years.

To assess the contribution of the cover crops to the SOC in the soil profile, isotopic abundance (^{13}C) was determined for samples from the contrasting treatments in the sub-subplots with no nitrogen fertilization: CT O/M, CT OV/MC, NT O/M and NT OV/MC. The isotopic abundance values for the 0–5, 20–30 and 75–100 cm soil layers were determined using an elemental analyzer (FlashEA 2000, Thermo Fisher Scientific, Bremem, Germany) coupled to an isotope ratio mass spectrometer (IRMS) (Delta Advantage, Thermo Fisher Scientific, Bremen, Germany).

The conversion rates of C input into SOC at 0–100 cm depth were calculated as the ratio between the additional SOC stock at 0–100 cm depth and C input derived from legume cover crops and N fertilization. For legume cover crops, the difference between SOC stocks of legume cover crops treatments (V/M and OV/MC) and O/M was divided by the difference of C input between the same treatments inside of each tillage system (NT or CT) for the 0 N treatment. Values for V/M and OV/MC were then averaged. For N fertilization, the difference between SOC stocks of 180 N and 0 N was divided by the difference of C input between the same treatments inside of each tillage system (NT or CT) for the O/M system, only.

2.3. Historical evaluation of SOC stocks and storage rates

We compared our results obtained in 2014 with those obtained in five previous studies conducted since the beginning of the field experiment to build a historical assessment of SOC evolution, specifically for the 0–20 cm layer, and for the 0 N treatment, which was the sampling depth and N level common to all studies. Previous SOC measurements were carried out in 1985 (Medeiros, 1988), in 1990 (Bayer and Mielniczuk, 1997), in 1994 (Bayer et al., 2000), in 1998 (Lovato et al., 2004), and in 2003 (Zanatta et al., 2007). The methodological approach for all these sampling dates was similar to that used in 2014, with the exception of the analytical C method. Therefore, SOC data from previous studies using the Walkley-Black analytical method (Nelson and Sommers, 1996) were corrected by a factor of 0.9422, which is the slope of the linear equation obtained by fitting the data provided by the two analytical methods (Walkley–Black and dry combustion) for more than 100 samples spanning a wide range of SOC contents (Zanatta et al., 2007). The SOC dataset for previous years was also recalculated from the original values using the equivalent soil mass method.

2.4. Statistical analyses

After normality was verified by means of the Kolmogorov-Smirnov test and homogeneity of variance by the Levene test, the SOC data were subjected to analysis of variance (ANOVA) and, significant results ($p < 0.05$) were compared using the Tukey test ($p < 0.05$). The MIXED procedure was used to compare the effects of tillage methods (T), crop systems (CC), nitrogen fertilization (N) and soil layers (L) on the response variables. This procedure considers the main factors and their interactions as fixed factors and the block variable and the experimental errors as random variables. Because the estimation of annual C input was based on evaluations performed on most of years but not always for all treatments, and in addition to the fact that C input from maize to have been indirectly estimated from grain yield, the C input data was not statistically analyzed.

All analyses were performed using the SAS statistical package® v.9.4 (Statistical Analysis System Institute, Cary, North Carolina). The statistical model used in the analysis of variance to evaluate C concentration was as follows:

$$Y_{ijklm} = \mu + B_i + T_j + Error_{(ij)} + C_k + T_jC_k + Error_{(ijk)} + N_l + T_jN_l + C_kN_l + T_jC_kN_l + Error_{(ijkl)} + L_m + Error_{(im)} + T_jL_m + C_kL_m + N_lL_m + T_jC_kL_m + T_jN_lL_m + C_kN_lL_m + T_jC_kN_lL_m + Error_{(ijklm)}.$$

Where µ = general mean of the experiment; B = block (i = 1–3); T = tillage systems (j = 1, 2); C = cropping system (k = 1–3); N = nitrogen fertilization (l = 1, 2); L = soil layers (m = 1–8), and Error = experimental error. To assess the SOC stocks and storage rates, the variable soil layers and its associated errors were removed from the statistical model.

Regression analyses were performed (SigmaPlot for Windows v. 12.0, Systat Software, Inc., San Jose, CA) to explore the relationships between SOC stocks and C inputs and the relationship between SOC storage rates in the 0–30 cm and 0–100 cm soil layers.

3. Results

3.1. Annual average of carbon input data

The inclusion of legume cover crops and nitrogen fertilization caused an approximately twofold increase in the annual C input, which amounted to 4.98 and 10.0 Mg ha^{-1} year^{-1} in the O/M 0 N and OV/MC 180 N systems (Fig. 1), respectively.

In systems where nitrogen fertilizer was applied to the maize crop, the impact of legume cover crops on C input was smaller because C inputs from maize were similar for the three cropping systems. In all cropping systems, maize made a significant contribution in terms of C input, representing between 42 and 67% of the total C input (Fig. 1).

3.2. C storage in the surface soil layer over time

Thirty years of contrasting management systems resulted in a large difference in SOC stocks in the 0–20 cm layer (Fig. 2a) of this previously degraded subtropical soil. From 1985 to 2014, in systems with no nitrogen fertilization, the conventional management system (CT O/M) resulted in a decrease of 3.8 Mg ha^{-1} in SOC stocks of the 0–20 cm layer, with a greater reduction in the first 5 years (Fig. 2a). In contrast, all systems with legume cover crops showed a positive SOC balance over the 30 years. In NT, the OV/MC system increased SOC stocks by 5.6 Mg ha^{-1} to 41.8 Mg ha^{-1} (Fig. 2a).

The SOC storage rate for the 20-cm soil layer reached its maximum level between five and nine years after the experiment began, reaching 1 Mg ha^{-1} year^{-1} in the NT OV/MC system (Fig. 2b). After this peak in SOC storage, the rates decreased exponentially over time. However, it is

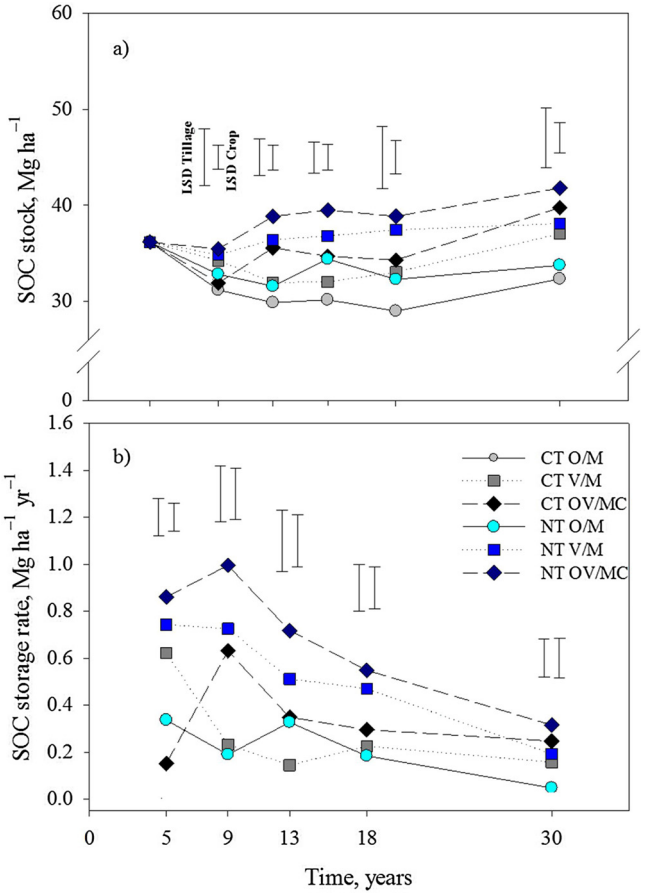

Fig. 2. Temporal evolution of (a) soil organic C (SOC) stocks, (b) SOC storage rates in the 0–20 cm of a subtropical Acrisol under two tillage systems (CT-conventional tillage and NT-no tillage) combined with three cropping systems [oat/maize (O/M), vetch/maize (V/M) and oat + vetch/maize + cowpea (OV/MC)], with no addition of N fertilization (0 N). The SOC storage rate was calculated relative to the reference CT O/M 0 N treatment (baseline). The vertical bars indicate the least significant difference (LSD) ($p < 0.05$).

noteworthy that even after 30 years, all systems continued to accumulate SOC in the 0 − 20 cm soil layer, at rates ranging from 0.05 to 0.32 Mg ha^{-1} year^{-1} according to the tillage and cropping systems.

3.3. Whole profile (0–100 cm) soil C stocks after 30 years

3.3.1. Tillage systems

The increase in SOC under NT occurred mainly in the surface soil layer (0–5 cm) and is also reflected in the higher SOC stocks observed in the 0–30 cm layer when compared to CT (Table 1). SOC stocks between tillage systems ranged from 47.9 to 63.3 Mg ha^{-1} in the 0–30 cm layer, and from 119.6 to 154.2 Mg ha^{-1} in the 0–100 cm layer (Table 1). The largest differences in the 0–100 cm layer between CT and NT were 13 and 11 Mg ha^{-1} in the O/M 180 N and OV/MC 180 N systems, respectively, which led to significant increments of 0.42 and 0.36 Mg ha^{-1} year^{-1} in SOC storage rates relative to the reference (CT O/M 0 N, Table 2).

3.3.2. Cropping systems

The use of two legume cover crops (one in winter and the other in summer - OV/MC) under NT increased SOC concentrations in the 0–10 cm layer by 39% compared to exclusive grass-type crops (O/M), and this increase explained the difference in SOC stocks in the 0–30 cm layer (Table 1). In the 0–100 cm layer, OV/MC increased the SOC stocks by an average of 14 Mg C ha^{-1} relative to O/M (Table 1). When considering 180 N, and on average for CT 180 N and NT, the SOC storage rate was 0.38 Mg ha^{-1} year^{-1} greater under OV/MC than under O/M for the 0–100 cm layer (Table 2).

The $\delta^{13}C/^{12}C$ of the soil under natural vegetation varied between -15.9 and -14.4‰ owing to the predominance of C4 plants. A decrease (more negative values) in the carbon isotope signature was observed due to the contribution of C3 plants (oat, vetch and cowpea) to SOC during the 30 years of experiment in all treatments (Table 3). The $\delta^{13}C/^{12}C$ values were more negative under OV/MC than under O/M in the 0–5 and 75–100 cm soil layers.

3.3.3. Nitrogen fertilization

Nitrogen fertilization with urea at a rate of 180 kg ha^{-1} led to an average increase of 16% in SOC concentration in the first 10 cm of soil compared to unfertilized treatments (Table 1). This significant difference in SOC in the surface layer translated into a significant effect of fertilization on SOC stocks in both the 0–30 and 0–100-cm soil layers (Table 1). For instance, in CT O/M, SOC stocks increased from 119.6 Mg C ha^{-1} to 131.1 Mg C ha^{-1} (Table 1) due to addition of 180 kg ha^{-1} of N-urea, leading to a SOC storage rate of 0.38 Mg ha^{-1} year^{-1} (Table 2).

For both the 0–30 and 0–100 cm soil layers, annual C inputs explained between 68% and 85% of the SOC stock variation under NT and CT (Fig. 3a and b). The SOC storage rate was 62% higher when considering the whole soil profile (0–100 cm layer) compared to the 0–30 cm layer only (Fig. 4). Thirty years of NT and OV/MC with 180 kg ha^{-1} of N-urea resulted in a SOC storage rate that was 1.15 Mg ha^{-1} year^{-1} higher in the 0–100 cm depth compared to CT and O/M without nitrogen fertilization (Fig. 5). A marked effect on SOC was observed in the surface layers but also throughout the soil profile down to a depth of 100 cm.

On average, the additional SOC storage attributable to the use of legume cover crops was almost twice that induced by nitrogen fertilization (Fig. 6). In other words, each kg of C input induced by the use of legume cover crops resulted in an average conversion rate of 0.15 kg of SOC per Kg of C input (range of 0.08–0.39), compared to only 0.08 kg SOC per kg C input (range of 0.01–0.19) for nitrogen fertilization (Fig. 6).

4. Discussion

Thirty years of contrasting management systems resulted in large differences (up to 35 Mg ha^{-1}) in SOC stocks in the whole soil profile (0–100 cm) (Table 1) of this previously degraded subtropical Acrisol. As observed in previous studies (Boddey et al., 2010; Alburquerque et al., 2015; Miranda et al., 2016), the use of NT leads to significant SOC storage in the Southern Brazil environment, both in the surface and subsurface soil layers. In our study the tillage effect was not attributable to differences in C inputs as crop yields were similar between the two tillage treatments. However, within each tillage system, approximately 80% of the variation in SOC stocks was explained by C inputs as affected by cropping systems and N fertilization (Fig. 3a and b). Cropping systems which included legume cover crops increased SOC storage with the highest potential recorded when two legumes (OV/MC) were cultivated. When averaged over the whole experimental period, vetch and vetch + cowpea legume residues added 82 and 115 Kg N ha^{-1}yr^{-1}, respectively, which improved grain yield and biomass production of maize in the crop sequence (Lovato et al., 2004; Amado et al., 2006). In addition to favoring the supply of N and increasing C inputs from maize or a subsequent crop, legume cover crops also added C from their own residues (Lovato et al., 2004; Amado et al., 2006), which includes inputs from roots that likely affect the SOC in deeper soil layers. Nitrogen fertilization (at a rate of 180 kg ha^{-1}) also had a positive effect on SOC stocks due to the larger amount of residue (shoot + root) added by the plants.

The SOC storage per unit of C input induced by legume cover crops was on average about double of that induced by N fertilization (Fig. 6)

Table 1
SOC concentration in eight soil layers and SOC stocks in two soil layers of a sandy clay loamAcrisol subjected to conventional tillage (CT) and no-till (NT) for three cropping systems: oat/maize (O/M), vetch/maize (V/M) and oat + vetch/maize + cowpea (OV/MC); with two N-urea rates (0 N = 0 kg ha^{-1} and 180 N = 180 kg ha^{-1}).

Fertilization	Tillage	Crop	SOC concentration[a,b]								SOC stocks[c]	
			Soil layer (cm)									
			0–5 g kg^{-1}	5–10	10–15	15–20	20–30	30–50	50–75	75–100	0–30 Mg ha^{-1}	0–100
0 N	CT	O/M	10.6	9.5	9.4	9.8	9.1	8.7	6.4	5.4	47.9	119.6
		V/M	12.2	12.0	10.8	10.0	9.9	8.8	7.3	6.2	53.8	133.2
		OV/MC	13.4	11.7	12.6	10.6	10.0	9.2	8.3	6.6	56.7	142.9
	NT	O/M	15.0	8.9	8.8	8.8	10.6	9.7	8.4	6.5	51.7	139.5
		V/M	19.4	10.5	8.8	8.4	9.8	9.6	8.1	6.4	54.6	140.5
		OV/MC	20.9	11.7	9.8	9.4	10.0	9.9	8.5	6.7	58.6	148.2
180 N	CT	O/M	11.3	10.0	10.6	9.4	10.4	8.9	7.3	6.2	51.7	131.1
		V/M	14.1	12.1	10.4	9.2	9.9	9.4	7.8	6.1	54.4	137.1
		OV/MC	12.9	11.2	11.8	10.3	10.4	9.9	8.2	6.5	55.8	143.3
	NT	O/M	17.2	9.8	9.2	8.7	10.1	10.4	8.5	6.5	53.5	143.6
		V/M	22.9	12.0	9.1	8.2	10.3	9.8	8.1	6.4	59.4	146.1
		OV/MC	25.9	13.3	9.8	8.8	10.0	10.3	8.6	6.7	63.3	154.2
LSD ($p < 0.05$)		Tillage					1.4				5.6	12.9
		Crop					1.7				5.5	15.7
		N fertilization					1.1				3.8	7.2
		Layer					2.0				–	–

The least significant difference (LSD) ($p < 0.05$) was made considering two triple interaction for SOC concentration (see below numbers 1 and 2) and one triple interaction for the both SOC stocks 0–30 cm and 0–100 cm (see below number 3):

[a] Interaction between tillage and cropping systems and soil layers. Comparisons of SOC concentrations were performed on the average of two nitrogen fertilization rates.

[b] Interaction between tillage systems, nitrogen fertilization and soil layers. Comparisons of SOC concentrations were performed on the average of the three cropping systems.

[c] Interaction between tillage and cropping systems and nitrogen fertilization for comparisons of SOC stocks.

Table 2
SOC storage rates in a sandy clay loam Acrisol subjected to conventional tillage (CT) and no-till (NT) for three cropping systems: oat/maize (O/M), vetch/maize (V/M) and oat + vetch/maize + cowpea (OV/MC); with two N-urea rates (0 N = 0 kg ha^{-1} and 180 N = 180 kg ha^{-1}).

Fertilization	Tillage	Crop	SOC storage rate	
			0–30 Mg ha^{-1} yr^{-1}	0–100
0 N	CT	O/M	0.00	0.00
		V/M	0.15	0.42
		OV/MC	0.25	0.80
	NT	O/M	0.13	0.66
		V/M	0.22	0.70
		OV/MC	0.26	0.95
180 N	CT	O/M	0.13	0.38
		V/M	0.22	0.58
		OV/MC	0.36	0.79
	NT	O/M	0.19	0.80
		V/M	0.38	0.88
		OV/MC	0.51	1.15
LSD* ($p < 0.05$)		Tillage	0.18	0.36
		Crop	0.20	0.41
		N fertilization	0.15	0.20

* Least significant difference ($p < 0.05$) of interaction between tillage, cropping systems and nitrogen fertilization for comparisons of SOC storage rate.

Table 3
Natural abundance of ^{13}C in the 0–5, 20–30, and 75–100 cm layers of a sandy clay loam Acrisol subjected to conventional tillage (CT) and no-tillage (NT) with oat/maize (O/M) and oat + vetch/maize + cowpea (OV/MC) and no N fertilization.

Depth (cm)	δ $^{13}C/^{12}C$ (‰)			
	Tillage	Crop		NG
0–5				−15.9 ± 0.2[b]
	CT	O/M	−15.0 ± 1.0[a] Aa[2]	
		OV/MC	−18.9 ± 0.6 Ab	
	NT	O/M	−15.1 ± 0.7 Aa	
		OV/MC	−20.8 ± 0.4 Bb	
20–30				−14.4 ± 0.4
	CT	O/M	−15.4 ± 0.2 Aa	
		OV/MC	−15.8 ± 0.4 Aa	
	NT	O/M	−15.5 ± 0.8 Aa	
		OV/MC	−16.5 ± 0.9 Aa	
75–100				−14.8 ± 0.4
	CT	O/M	−14.9 ± 0.2 Aa	
		OV/MC	−18.4 ± 0.6 Bb	
	NT	O/M	−15.0 ± 0.3 Aa	
		OV/MC	−16.7 ± 0.8 Ab	

[a] Standard deviation.

[b] Uppercase letters compare tillage systems and lowercase letters compare cropping systems; means with the same letter do not differ significantly according to Tukey test ($p < 0.05$).

which suggests a possible effect of residue quality. Compared to grass species, the greater lability of the legume cover crop residues may contribute more microbial residues, which are stabilized by chemical bonding with the mineral soil matrix (Cotrufo et al., 2013, 2015). This difference could also be attributable to mineral N fertilization inducing accelerated C mineralization (Khan et al., 2007), although this mechanism is considered controversial (Powlson et al., 2010).

Considering only the surface soil layer (0–20 cm Fig. 2b), the pattern of high initial rates of SOC storage (first 5–9 years) and the subsequent decline would at first have suggested that the adoption of conservation management systems in a previously degraded soil could be seen as a limited short-term strategy for removing CO_2 from the

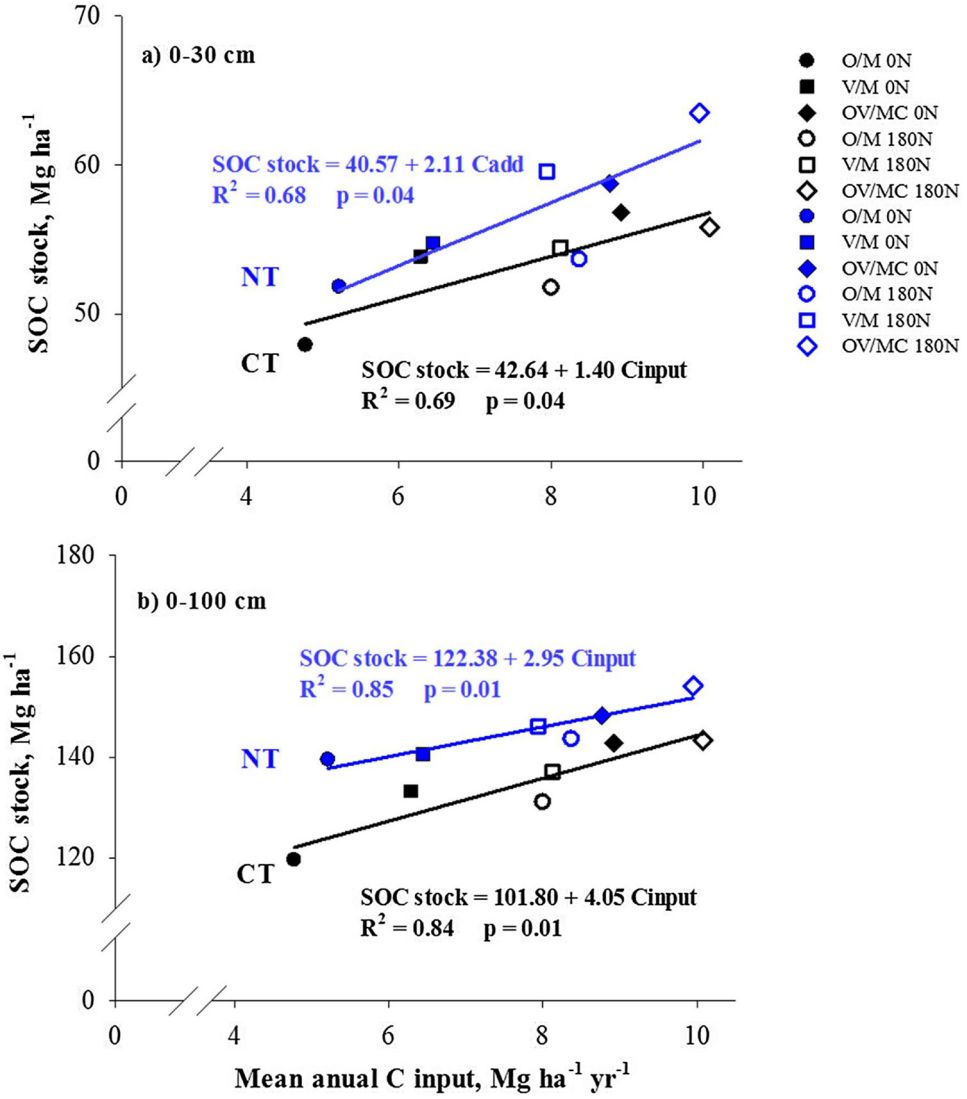
a) 0-30 cm
SOC stock = 40.57 + 2.11 Cadd
R² = 0.68 p = 0.04
NT
CT
SOC stock = 42.64 + 1.40 Cinput
R² = 0.69 p = 0.04
SOC stock, Mg ha-1
b) 0-100 cm
SOC stock = 122.38 + 2.95 Cinput
R² = 0.85 p = 0.01
SOC stock = 101.80 + 4.05 Cinput
R² = 0.84 p = 0.01
Mean anual C input, Mg ha-1 yr-1
O/M 0N
V/M 0N
OV/MC 0N
O/M 180N
V/M 180N
OV/MC 180N

Fig. 3. Relationship between soil organic C (SOC) stocks in the 0–30 and 0–100 cm soil layers of a subtropical Acrisol and annual C addition by cropping systems [oat/maize (O/M), vetch/maize (V/M) and oat + vetch/maize + cowpea (OV/MC)] subjected to two N-urea rates, 0 kg ha^{-1} (0 N) and 180 kg ha^{-1} (180 N), under conventional tillage (CT) and no-tillage (NT) systems.

atmosphere. However, more than half of the SOC storage after 30 years was attributable to the increase in C stocks in the subsurface layer (30–100 cm) (Fig. 4). Based on the carbon isotope signature, C storage in the deeper layers can be at least partly ascribed to the cover crop residues (shoot and root). Indeed, the more negative values in the 75–100 cm soil layer (Table 3) of the OV/MC system relative to O/M are due to the greater contribution of residues of C3 plants (oats, vetch and cowpea) which favored SOC accumulation.

Our results showing substantial additional SOC storage in deep soil layers (below 30 cm) induced by conservation management agree with the findings of others studies conducted in tropical (Miranda et al., 2016) and subtropical (Boddey et al., 2010; Alburquerque et al., 2015) regions of Brazil. However, they contrast with the results of studies conducted in temperate regions (Baker et al., 2007; Dimassi et al., 2014). This difference between regions can be due to various factors, like differences in residue inputs (shoot and root), rainfall patterns, and soil type. In European regions with rainfall levels varying between 350 and 800 mm year^{-1}, Dimassi et al. (2014) found a negative relationship between the increase in SOC stocks in NT vs. CT and annual precipitation. This precipitation level is well below the mean rainfall of the region where the present study was carried out (1440 mm year^{-1}). This higher rainfall, taken together with the more labile material left on the NT surface, may favor the percolation of dissolved organic carbon (DOC) into the soil profile (Sanderman et al., 2008; Kaiser and Kalbitz, 2012).

In addition, the lighter texture of the soil in the surface layer may have promoted rainwater percolation and, consequently, DOC translocation into the soil profile (Wang et al., 2015). C sorption on minerals may be more efficient in the subsurface than in surface layers because the greater C saturation deficit (Castellano et al., 2015). This also shows the importance of cropping systems in terms of providing residues potentially contributing to COD supply and resulting in their accumulation in deeper soil layers, especially in soils whose clay content increases deeper in the soil profile (Torres-Sallan et al., 2018). Furthermore, the oxide mineralogy of Acrisol is conducive to strong interactions with organic matter, leading to C stabilization (Denef et al., 2004; Boddey et al., 2010).

Our results show that, under the humid subtropical climate conditions of southern Brazil, a high potential exists for the storage of SOC along the soil profile. Considering the 0–100 cm soil profile and compared to our reference system (CT O/M 0 N), 30 years of alternative management systems resulted in high SOC storage rates (range from 0.38 to 1.15 Mg C ha^{-1} year^{-1}, Table 2). Thus, our results support the idea that soil carbon accounting systems should consider soil layers

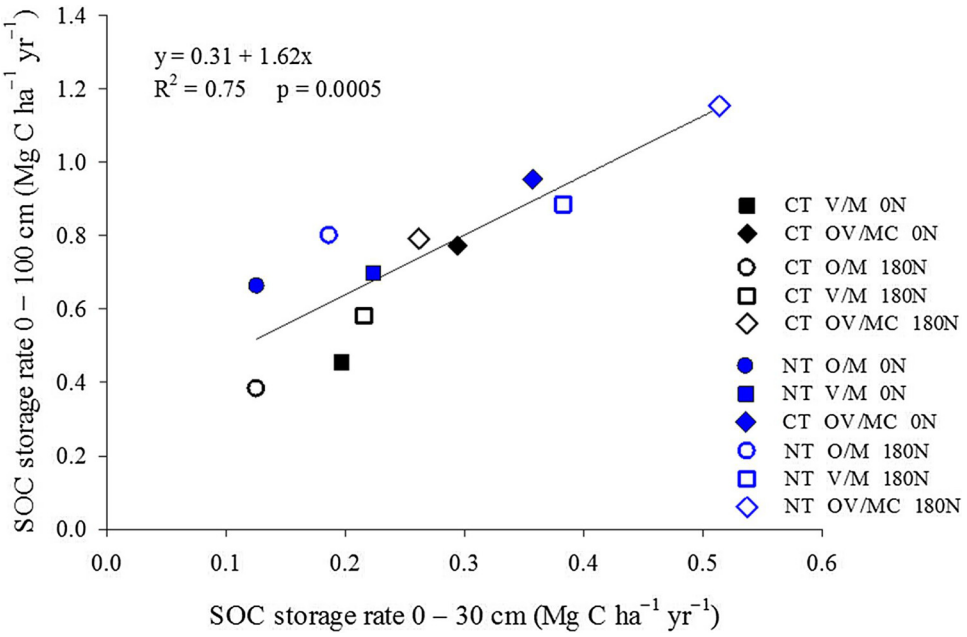
y = 0.31 + 1.62x
R2 = 0.75 p = 0.0005
SOC storage rate 0 – 100 cm (Mg C ha-1 yr-1)
SOC storage rate 0 – 30 cm (Mg C ha-1 yr-1)
CT V/M 0N
CT OV/MC 0N
CT O/M 180N
CT V/M 180N
CT OV/MC 180N
NT O/M 0N
NT V/M 0N
CT OV/MC 0N
NT O/M 180N
NT V/M 180N
NT OV/MC 180N

Fig. 4. Relationship between SOC storage rates in the 0–30 cm and storage rates in the 0–100 cm soil layers under conventional tillage (CT) and no-tillage (NT) combined with three cropping systems [oat/maize (O/M), vetch/maize (V/M) and oat + vetch/maize + cowpea (OV/MC)]; with two N-urea rates (0 N = 0 kg ha^{-1} e 180 N = 180 kg ha^{-1}).

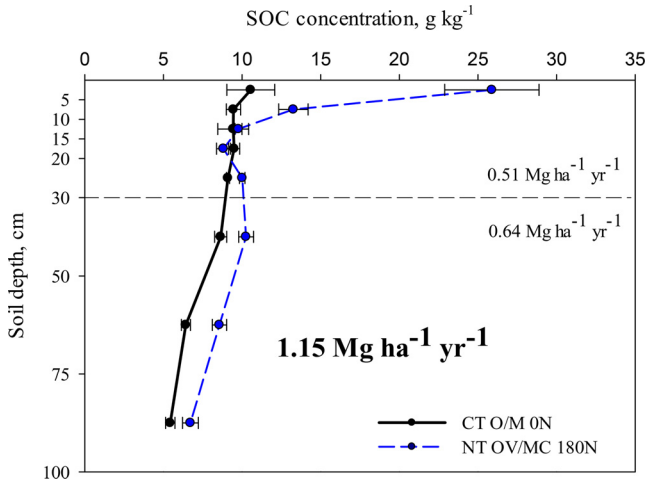

Fig. 5. Concentration of SOC at 0–100 cm depth in a subtropical Acrisol under NT OV/MC 180 N compared to CT O/M 0 N. The values 0.51, 0.65 and 1.15 Mg ha^{-1} yr^{-1} represent the SOC storage rate in the 0–30, 30–100 and 0–100 cm soil layers, respectively, under NT OV/MC 180 N considering CT O/M 0N as a reference. The bars represent standard errors.

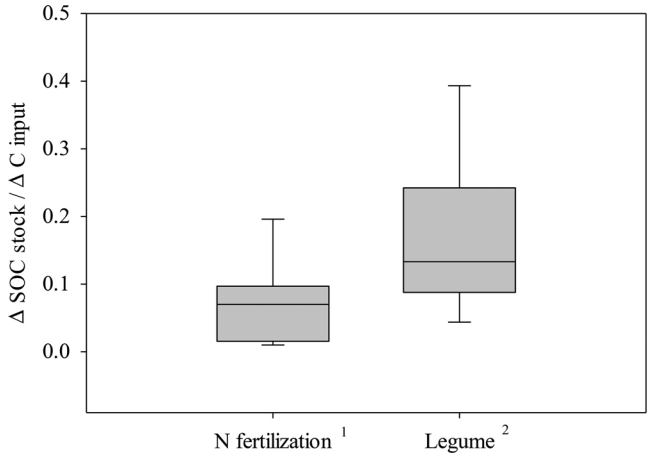

Fig. 6. Conversion rate of C input into SOC at 0–100 cm depth in a subtropical Acrisol under N fertilization and legume cover cover crops.
[1] [(SOC stocks of 180 N - SOC stocks of 0 N)/(C input of 180 N – C input of 0 N)] calculated for O/M cropping system, and averaged for the two tillage systems (CT and NT).
[2] [(SOC stocks of V/M or OV/MC - SOC stocks of O/M)/(C input of V/M or OV/MC – C input of O/M)], averaged for the two legume cropping systems (V/M and OV/MC) and for the two tillage systems (CT or NT).

deeper than 30 cm to fully account for management-induced changes.

The potential effect of NT associated with legume crops on SOC storage is an avenue that Brazil could pursue to meet its GHG emissions reduction target. At the Paris Convention of the Parties (COP21) in December 2015, the French government proposed the "4 per 1000" initiative "Soils for Food Security and Climate" (https://www.4p1000.org/). Considering Brazil's mean SOC stocks of 45 Mg C ha^{-1} in the 0–30 cm soil layer (Fidalgo et al., 2007), its target under the 4 per mille initiative would correspond to a SOC storage rate of 0.18 Mg C ha^{-1} year^{-1}. This target has been shown to be attainable with the adoption of conservation management systems compared to more conventional systems, such as the adoption of two legume cover crops in NT in our study (0.76 Mg C ha^{-1} year^{-1}).

The proposed conversion of 8 million hectares from CT to NT over the next few years in Brazil represents potential soil C storage of about 8 Tg C year^{-1} (MAPA, 2012). Half of the area under NT in Brazil (16 Mha) is currently devoted to monocultures. Since the adoption of more diversified cropping systems could provide additional storage of 0.35 Mg C ha^{-1} year^{-1} (difference in SOC storage rate between NT O/M 180 N and NT OV/MC 180 N), the resulting improvement in the management system could result in an additional SOC storage of 5.6 Tg C year^{-1}. With the conversion of cropping areas to NT and the adoption of legume cover crops under NT, a total soil carbon sequestration capacity of 50 Tg CO_2 year^{-1} could technically be attained in Brazil. Thus, if we consider improved soil management systems, relative to more traditional systems, in Brazil alone, this would mean off-setting total CO_2 emissions by 11% [50 Tg CO_2 yr^{-1} divided by 466 Tg CO_2 yr^{-1} released from Brazilian agriculture activities according to Lapola et al. (2014)], and hence contribute significantly to the target set for Brazil 2025 (37% reduction by 2025) established at the Paris climate change conference.

The net benefits of conservation management systems in terms of atmospheric C removal in soil should be complemented with studies involving a global greenhouse gas balance. In fact, some recent studies

in Brazil have shown a limited impact of practices such as no-tillage and legume cover crops on N_2O emissions, the effect of which was surpassed by the resulting SOC storage (Bayer et al., 2016; Piva et al., 2012). However, the contribution of N fertilization to GHG mitigation requires a more thorough assessment owing to its strong impact on N_2O emissions.

5. Conclusion

Our study examined the effect of no-tillage, legume cover crops, and nitrogen (N) fertilization from the perspective of potential SOC storage in surface and subsurface soil layer of a previously degraded subtropical Acrisol. The results indicate that no-tillage with high and diversified residue inputs from legume cover crops is an effective long-term measure for SOC storage and potential mitigation of global warming in tropical and subtropical conditions in comparison with traditionally adopted farming practices in Brazil (conventional tillage without legume cover crops and N fertilization). Although the rate of SOC storage in surface layers decreases over time, conservation management systems favored SOC accumulation in subsurface layers in the studied subtropical soil, contributing to maintain high SOC storage rates in the system during 30 years.

Acknowledgments

The authors are grateful to the staff of the Experimental Station and Department of Soil Science of the Federal University of Rio Grande do Sul for their help with field and laboratory activities. They also thank the National Council for Scientific and Technological Development (CNPq) and Foundation for Research Support of Rio Grande do Sul State (Fapergs) for funding this work and maintaining the long-term field experiment, and the Coordination for the Improvement of Higher Education Personnel (CAPES) for awarding M. G. Veloso a “sandwich” doctorate scholarship tenured at Agriculture and Agri-Food Canada, Québec.

References

Alburquerque, M.A., Dieckow, J., Sordi, A., Piva, J.T., Bayer, C., Molin, R., Pergher, M., Ribeiro-Júnior, P.J., 2015. Carbon and nitrogen in a Ferralsol under zero-tillage rotations based on cover, cash or hay crops. Soil Use Manage. 31, 1–9. https://doi.org/10.1111/sum.12173.

Amado, T.J., Bayer, C., Conceição, P.C., Spagnollo, E., de Campos, B.H., da Veiga, M., 2006. Potential of carbon accumulation in no-till soils with intensive use and cover crops in southern Brazil. J. Environ. Qual. 35, 1599–1607. https://doi.org/10.2134/jeq2005.0233.

Angers, D., Bolinder, M.A., Carter, M.R., Gregorich, E.G., Drury, C.F., Liang, B.C., Voroney, R.P., Simard, R.R., Donald, R.G., Beyaert, R.P., Martel, J., 1997. Impact of tillage practices on organic carbon and nitrogen storage in cool, humid soils of eastern Canada. Soil Tillage Res 41, 191–201. https://doi.org/10.1016/S0167-1987(96)01100-2.

Baker, J.M., Ochsner, T.E., Venterea, R.T., Griffis, T.J., 2007. Tillage and soil carbon sequestration – what do we really know? Agric. Ecosyst. Environ. 118, 1–5. https://doi.org/10.1016/j.agee.2006.05.014.

Bayer, C., Gomes, J., Zanatta, J.A., Vieira, F.C.B., Dieckow, J., 2016. Mitigating greenhouse gas emissions from a subtropical ultisol by using long-term no-tillage in combination with legume cover crops. Soil Tillage Res. 161, 86–94. https://doi.org/10.1016/j.still.2016.03.011.

Bayer, C., Mielniczuk, J., 1997. Características químicas do solo afetadas por métodos de preparo e sistemas de cultura. Rev. Bras. Cienc. Solo 21, 105–112.

Bayer, C., Mielniczuk, J., Amado, T.J.C., Martin-Neto, L., Fernandes, S.V., 2000. Organic matter storage in a sandy clay loam Acrisol affected by tillage and cropping systems in southern Brazil. Soil Tillage Res. 54, 101–109. https://doi.org/10.1016/s0167-1987(00)00090-8.

Bayer, C., Martin-Neto, L., Mielniczuk, J., Pillon, C.N., Sangoi, L., 2001. Changes in soil organic matter fractions under subtropical no-till cropping systems. Soil Sci. Soc. Am. J. 65, 1473–1478.

Bayer, C., Martin-Neto, L., Mielniczuk, J., Pavinato, A., Dieckow, J., 2006. Carbon sequestration in two Brazilian Cerrado soils under no-till. Soil Tillage Res. 86, 237–245. https://doi.org/10.1016/j.still.2005.02.023.

Blake, G.R., Hartge, K.H., 1986. Bulk density. In: Klute, A. (Ed.), Methods of Soil Analysis, Part 1. Physical and Mineralogical Methods. ASA/SSSA, Madison, pp. 363–382.

Blanco-Canqui, H., Schlegel, A.J., Heer, W.F., 2011. Soil-profile distribution of carbon and associated properties in no-till along a precipitation gradient in the central Great Plains. Agric. Ecosyst. Environ. 144, 107–116. https://doi.org/10.1016/j.agee.2011.07.004.

Boddey, R.M., Jantalia, C.P., Conceição, P.C., Zanatta, J.A., Bayer, C., Mielniczuk, J., Dieckow, J., Santos, H.P., Denardin, J.E., Aita, C., Giacomini, S.J., Alves, B., Urquiaga, S., 2010. Carbon accumulation at depth in Ferralsols under zero-till subtropical agriculture. Glob. Change Biol. 16, 784–795. https://doi.org/10.1111/j.1365-2486.2009.02020.x.

Brazil Ministry of Environment, 2015. Intended Nationally Determined Contributions, iNDC BRASIL. Available in:. (Accessed 01 September 2017). http://www.mma.gov.br/images/arquivo/80108/BRAZIL%20iNDC%20english%20FINAL.pdf.

Castellano, M.J., Mueller, K.E., Olk, D.C., Sawyer, J.E., 2015. Integrating plant litter quality, soil organic matter stabilization, and the carbon saturation concept. Glob. Change Biol. 21, 3200–3209. https://doi.org/10.1111/gcb.12982.

Cotrufo, M.F., Wallenstein, M.D., Boot, C.M., Denef, K., Paul, E., 2013. The Microbial Efficiency-Matrix Stabilization (MEMS) framework integrates plant litter decomposition with soil organic matter stabilization: do labile plant inputs form stable soil organic matter? Glob. Change Biol. 19, 988–995. https://doi.org/10.1111/gcb.12113.

Cotrufo, M.F., Soong, J.L., Horton, A.J., Campbell, E.E., Haddix, M.L., Wall, D.H., Parton, W.J., 2015. Formation of soil organic matter via biochemical and physical pathways of litter mass loss. Nat. Geosci. 8, 776–779. https://doi.org/10.1038/NGEO2520.

Denef, K., Six, J., Merckx, R., Paustian, K., 2004. Carbon sequestration in microaggreagtes of no-tillage soils with different clay mineralogy. Soil Sci. Soc. Am. J. 68, 1935–1944.

Diekow, J., Mielniczuk, J., Knicker, H., Bayer, C., Dick, D.P., Kogel-Knabner, I., 2005. Soil C and N stocks as affected by cropping systems and nitrogen fertilisation in a southern Brazil Acrisol managed under no-tillage for 17 years. Soil Tillage Res. 81, 87–95. https://doi.org/10.1016/j.still.2004.05.003.

Dimassi, B., Mary, B., Wylleman, R., Labreuche, J., Couture, D., Piraux, F., Cohan, J.P., 2014. Long-term effect of contrasted tillage and crop management on soil carbon dynamics during 41 years. Agric. Ecosyst. Environ. 188, 136–146. https://doi.org/10.1016/j.agee.2014.02.014.

Ellert, B.H., Bettany, J.R., 1995. Calculation of organic matter and nutrients stored in soils under contrasting management regimes. Can. J. Soil Sci. 75, 529–538.

FAO, 2002. World Reference Base for Soil Resources. FAO, Rome.

Fidalgo, E.C.C., Benites, V.M., Machado, P.L.O.A., Madari, B.E., Coelho, M.R., Moura, I.B., Lima, C.X., 2007. Estoque de carbono nos solos do Brasil. Embrapa Solos, Rio de Janeiro 26 p.

Hobley, E.U., Wilson, B., 2016. The depth distribution of organic carbon in the soils of eastern Australia. Ecosphere 7, e01214. https://doi.org/10.1002/ecs2.1214.

Kaiser, K., Kalbitz, K., 2012. Cycling downwards – dissolved organic matter in soils. Soil Biol. Biochem. 52, 29–32 doi: 0038-0717.

Khan, S.A., Mulvaney, R.L., Ellsworth, T.R., Boast, C.W., 2007. The myth of N fertilization to soil organic carbon sequestration. J. Environ. Qual. 36, 1821–1832. https://doi.org/10.2134/jeq2007.0099.

Kirchmann, H., Schon, M., Borjesson, G., Hamner, K., Katterer, T., 2013. Properties of soils in the Swedish long-term fertility experiments: VII. Changes in topsoil and upper subsoil at orja and Fors after 50 years of nitrogen fertilization and manure application. Acta Agric. Scand. Sect. B–Soil Plant Sci. 63, 25–36. https://doi.org/10.1080/09064710.2012.711352.

Lal, T., Reicosky, D.L., Hanson, J.D., 2007. Evolution of the plow over 10,000 years and the rationale for no-till farming. Soil Tillage Res. 93, 1–12. https://doi.org/10.1016/j.still.2006.11.004.

Lapola, D.M., Martinelli, L.A., Peres, C.A., Ometto, J.P.H.B., Ferreira, M.E., Nobre, C.A., Aguiar, A.P.D., Bustamante, M.M.C., Cardoso, M.F., Costa, M.H., Joly, C.A., Leite, C.C., Moutinho, P., Sampaio, G., Strassburg, B.B.N., Vieira, I.C.G., 2014. Pervasive transition of the Brazilian land-use system. Nat. Clim. Change 4, 27–35. https://doi.org/10.1038/nclimate2056.

Lawrence, C.R., Harden, J.W., Xu, X., Marjorie, S., Schulza, M.S., Trumbore, S.E., 2015. Long-term controls on soil organic carbon with depth and time: a case study from the Cowlitz River Chronosequence, WA USA. Geoderma 247–248, 73–87. https://doi.org/10.1016/j.geoderma.2015.02.005.

Lovato, T., Mielniczuk, J., Bayer, C., Vezzani, F., 2004. Carbon and nitrogen addition related to stocks of these elements in soil and corn yield under management systems. Rev. Bras. Cienc. Solo 28, 175–187. https://doi.org/10.1590/s0100-06832004000100017.

Mack, M.C., Schuur, E.A.G., Bret-Harte, M.S., Shaver, G.R., Chapin, F.S., 2004. Ecosystem carbon storage in arctic tundra reduced by long-term nutrient fertilization. Nature 431, 440–443. https://doi.org/10.1038/nature02887.

Martins, M.M., Angers, D., Corá, J.E., 2012. Co-accumulation of microbial residues and particulate organic matter in the surface layer of a no-till oxisol under different crops. Soil Biol. Biochem. 50, 208–213. https://doi.org/10.1016/j.soilbio.2012.03.024.

MAPA, 2012. Plano Setorial de Mitigação e de Adaptação às Mudanças Climáticas para a Consolidação de uma Economia de Baixa Emissão de Carbono na Agricultura: Plano ABC (Agricultura de Baixa Emissão de Carbono). Ministério da Agricultura, Pecuária e Abastecimento, Ministério do Desenvolvimento Agrário, Coordenação da Casa Civil da Presidência da República, Brasília 173 p.

Medeiros, J.C., 1988. Sistemas de culturas adaptados a produtividade, recuperação e conservação do solo (M.Sc. Dissertation). Universidade Federal do Rio Grande do Sul, Porto Alegre/RS, Brazil.

Mielniczuk, J., Bayer, C., Vezzani, F.M., Lovato, T., Fernandes, F.F., Debarba, L., 2003. Manejo de solo e culturas e sua relação com os estoques de carbono e nitrogênio do solo. In: Curi, N., Marques, J.J., Guilherme, L.R.G., Lima, J.M., Lopes, A.S., Alvarez, V., V.H (Eds.), Tópicos em Ciência do solo 3, pp. 209–248 Viçosa.

Miranda, E., Carmo, J., Couto, E., Camargo, P., 2016. Long-term changes in soil carbon stocks in the Brazilian Cerrado under commercial soybean. Land Degrad. Dev. 27, 1586–1594. https://doi.org/10.1002/ldr.2473.

Nelson, P.W., Sommers, C.E., 1996. Total C, organic C and organic matter. In: Page, A.L. (Ed.), Methods of Soil Analysis (chemical). SSSA, Madison, USA, pp. 539–579.

Paustian, K., Andren, O., Janzen, H.H., Lal, R., Smith, P., Tian, G., Tiessen, H., Van Noordwijk, M., Woomer, P.L., 1997. Agricultural soils as a sink to mitigate CO_2 emissions. Soil Use Manage. 13, 230–244. https://doi.org/10.1111/j.1475-2743.1997.tb00594.x.

Piva, J.T., Dieckow, J., Bayer, C., Zanatta, J.A., de Moraes, A., Pauletti, V., Tomazi, M., Pergher, M., 2012. No-till reduces global warming potential in a subtropical ferralsol. Plant Soil 361, 359–373. https://doi.org/10.1007/s11104-012-1244-1.

Powlson, D.S., Jenkinson, D.S., Johnston, A.E., Poulton, P.R., Glendining, M.J., Goulding, K.W.T., 2010. Comments on "Synthetic nitrogen fertilizers deplete soil nitrogen: a global dilemma for sustainable cereal production" by Mulvaney, R.L., Khan, S.A., Ellsworth, T.R. in the Journal of Environmental Quality 2009 38: 2295–2314. J. Environ. Qual. 39, 749–752. https://doi.org/10.2134/jeq2008.0527.

Raphael, J.P., Calonego, J.C., Milori, D.M.B., Rosolem, C.A., 2016. Soil organic matter in crop rotations under no-till. Soil Tillage Res. 155, 45–53. https://doi.org/10.1016/j.still.2015.07.020.

Rogelj, J., Elzen, M., Höhne, N., Fransen, T., Fekete, H., Winkler, H., Schaeffer, R., Sha, F., Riachi, K., Meinshausen, M., 2016. Paris agreement climate proposals need a boost to keep warming well below 2 ºC. Nature 534, 631–639. https://doi.org/10.1038/nature18307.

Sanderman, J., Baldock, J.A., Amundson, R., 2008. Dissolved organic carbon chemistry and dynamics in contrasting forest and grassland soils. Biogeochemistry 89, 181–198. https://doi.org/10.1007/s10533-008-9211-x.

Torres-Sallan, G., Schulte, R.P.O., Lanigan, G.J., Byrne, K.A., Reidy, B., Simó, I., Six, J., Creamer, R.E., 2018. Clay illuviation provides a long-term sink for C sequestration in subsoils. Sci. Rep. 7, 45635. https://doi.org/10.1038/srep45635.

Wang, Y., Shao, M., Zhang, C., Liu, Z., Zou, J., Xiao, J., 2015. Soil organic carbon in deep profiles under Chinese continental monsoon climate and its relations with land uses. Ecol. Eng. 82, 361–367. https://doi.org/10.1016/j.ecoleng.2015.05.004.

Zanatta, J.A., Bayer, C., Dieckow, J., Vieira, F.C.B., Mielniczuk, J., 2007. Soil organic carbon accumulation and carbon costs related to tillage, cropping systems and nitrogen fertilization in a subtropical Acrisol. Soil Tillage Res. 94, 510–519. https://doi.org/10.1016/j.still.2006.10.003.